Supplementary information

Background

Only RIDL males were released in this experiment (see Methods), for which there are several compelling reasons[1-2]. One is that only female mosquitoes bite; another is that co-released sterile females may 'distract' sterile males from seeking out, courting and mating wild females[1,3]. Sterile-male-release methods require that released males survive in the field, disperse, find females and successfully court and inseminate them in competition with wild males; few field data are available regarding the ability of lab-reared, engineered males to achieve this[4-5]. Performance might be adversely affected by environmental aspects of rearing and distribution, by selection and drift associated with colonisation and laboratory rearing, and by the transgene itself[6-10]. With the OX513A strain subject to these factors in its development, rearing and field activities, the outcome of the experiment described was determined by the cumulative effect of these factors.

The stated sterile:wild adult male ratio target of 10:1 was based on SIT practice, though somewhat conservative relative to model predictions for fully competitive males[11-12]. Mortality in the RIDL strain, OX513A, generally occurs at a late larval stage[12]. For species with significant density-dependence at immature stages, such as *Aedes aegypti*[13], late-acting lethality is predicted to be more effective for population suppression than early-acting lethality[11-12]. Preliminary analysis of likely cost and effectiveness of large-scale use of release of RIDL males to control *Ae. aegypti* and dengue has indicated that this is likely to be an attractive method on both cost and effectiveness grounds[11,14-16]. Conventional SIT programs have been successfully operated on scales from very small[17] to vast, e.g. the continental-scale elimination of the New World screwworm *Cochliomyia hominivorax*[18-19]. Though no mosquito sterile-insect program has been operated on such a scale, successful precedents exist using chemicals or bacteria to effect sterilization[20-24]; *Ae. aegypti* is a relatively robust, easy-to-rear mosquito, so there are no obvious technical barriers to scale-up. Initial cost and effectiveness analyses have tended for simplicity to focus on the use of RIDL males as a stand-alone method, as indeed does the present study. However, in practice sterile-male methods are best used as components of an integrated vector management program, for example combining with community-based source reduction and larviciding. Such a program would enable the optimal exploitation of the different strengths and cost profiles of different methods, and should in general provide a more cost-effective solution than any component as a stand-alone method. In some cases there may be additional synergies, for example in respect of resistance management. If an effective dengue vaccine becomes widely available, a broad-based 'integrated disease management' approach, comprising a substantial vector control element, is still expected to be needed (e.g. Cuauhtemoc Ruiz Matus, PAHO, cited in[25] "An integrated approach to prevention and control is needed and a successful vaccine will be only a part of an integrated solution, complementing good vector control.")

Supplementary results and discussion

The magnitude of sex ratio change as a result of input of males indicates the OX513A:wild male ratio, assuming the wild and OX513A males are attracted equally to

the traps. The untreated areas, in Bodden Town, provided an indication of the likely sex ratio capture of wild *Ae. aegypti* without the effect of released OX513A males. Mosquitoes recovered in BG-Sentinel adult traps in untreated areas had a mean male:female ratio of 0.54:1 (95% bootstrap CI: 0.46:1 – 0.62:1; n=1637).

From field monitoring during RIDL releases, in parallel with increased OX513A:wild-type male ratio we observed an increase in the proportion of the field-collected eggs carrying the fluorescent marker, to a peak of 88% (**Fig. 2b**). This implies that the majority of wild females were mating OX513A males. Note that the increase in progeny fluorescence ratio appears to lag slightly behind the increase in release rates, as expected; a 5-day lag gives the highest estimated correlation, 0.90, between the release rates and the proportions of field-collected eggs carrying the fluorescent marker. For reference, the average life expectancy of *Aedes aegypti* females is generally taken to be about 7-9 days, based on a constant daily survival probability of 0.88-0.89[11,13,26-27] (but see also[28-30]). Average life expectancy estimates for males include 1-2 or 1.8-2.7 days for lab-colonized males[31-32] and various estimates between 1.6 and 3.8 days for wild males[32-36]. The subsequent reduction in fluorescence ratio (**Fig 2b**) may relate to the substantial suppression of the population at this stage (**Fig 2c** and below); lack of females in the treated area may mean that a higher proportion of eggs are laid by immigrants who may have mated before entering the release area. *Ae. aegypti* females who mate only once will continue to lay wild type eggs despite the presence of OX513A males. Monogamy[37-38], or at least first-male paternity[39], is thought to be typical for *Aedes aegypti*, though females have been reported to remate after inadequate semen transfer[40] or passage through one or more gonotrophic cycles[41]. A recent study[42] revisited the question of polyandry in large field cages, using stable-isotope labelling of males, and found 14% of females had engaged in multiple matings.

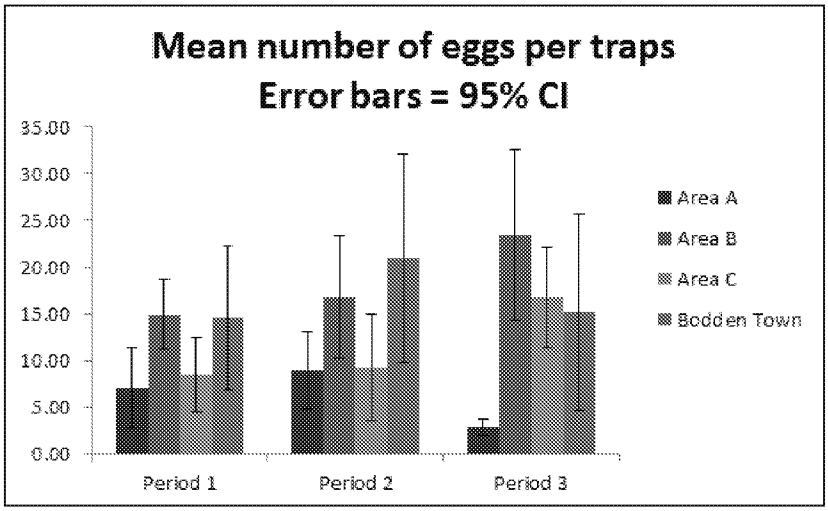
By comparing the RIDL:wild type male ratio with the progeny fluorescence ratio we can estimate the relative competitiveness of the two types of male. We have only attempted this for Period 2, as the low numbers of males in Period 1 and of eggs in Period 3 make further analysis of limited value. We estimate a relative competitiveness of 0.059 (95% bootstrap CI: 0.011 – 0.21) in Period 2. Production and male quality improved in Period 3 but insufficient eggs were recovered to allow a useful estimate of competitiveness. For comparison, in large-scale, successful SIT programs, field competitiveness of sterile males was estimated at 0.1 for New World screwworm (*C. hominivorax*)[43-44] and <0.01 for Mediterranean fruit fly (*Ceratitis capitata*)[3,45].

We used ovitrap index – the proportion of ovitraps in each area with one or more eggs after one week – as our primary measure of population density. Ovitraps compete with natural breeding sites for female oviposition. Ovitrap index is widely used as a measure of population density, though it is affected by the availability of alternative breeding sites, which is in turn affected by environmental factors such as rainfall[46]. However, our sites are close enough to share many environmental factors, and changes in ovitrap index over time and between sites should be a good indicator of changes in the mosquito population (**Fig. 2c**). Until early August, the weekly ovitrap indices for Area A were very similar to those in Areas C (likelihood ratio (LR) p=0.63) and B (LR p=0.13) but were significantly lower than in an external untreated control site, Bodden Town (LR p=0.026, roughly 40% lower). From early August onward the index in Area A was highly

significantly lower than that in other areas ($p<0.0001$ for each pairwise comparison; roughly 70% lower than Area C and Bodden Town through 16 December, and roughly 60% lower than Area B through 14 October; 80% lower than C for last 7 weeks of release period).

This may underestimate the underlying difference in oviposition and hence female numbers and the degree of suppression achieved as the ovitrap index is not a linear measure of female numbers. At high female numbers additional females will lay eggs in a trap already visited by a first female. Using a simple model in which females are assumed to lay at random in traps, the relative abundance or ratio (R) of two populations that give ovitrap indices of X and Y is $R = \log(1-X) / \log(1-Y)$. Using this formula, the difference between Areas A and C in the last seven weeks of the release period (ovitrap indices of 10% and 50% respectively) would correspond to a 6.6-fold rather than 5-fold difference in the female population, and hence 85% rather than 80% suppression of the target population relative to control.

We used ovitrap index as our primary measure, however alternative indices could be considered for example eggs/trap. This might be expected to be more linearly related to female numbers. However, we found the number of eggs per trap to be extremely variable, a problem compounded by the difficulty of achieving an accurate count at high egg numbers in a given trap. Mean eggs/trap are shown in **Suppl Fig 1** for the three release periods. In Periods 1 and 2 they are not significantly different between Areas A and C, whereas for Period 3 the mean for Area A (2.86 eggs/trap) is 17% that of Area C (16.8 eggs/trap). This is consistent with the 80% reduction inferred from uncorrected ovitrap index and 85% from the simple correction above.



Supplementary Figure 1. Eggs per trap in different areas and periods. Consistent with the ovitrap index data (**Fig. 2c**), Areas A and C have similar mean eggs per trap in Periods 1 and 2; in Period 3 Area A has lower mena eggs per trap (83% lower than Area C).

While it is clear that dengue cannot be transmitted in the absence of a suitable vector mosquito population, the relationship between (non-zero) vector population density and disease burden is much less clear. Thresholds for epidemic transmission, calculated from simulation models, imply that reductions of 10-90% from extant densities of *Ae. aegypti*

would be sufficient to prevent epidemic transmission of dengue in a range of settings[47]. The degree of suppression of the *Ae. aegypti* population observed even in this small trial against a non-isolated population would therefore likely be epidemiologically useful in many transmission settings; one might expect immigration to have much less of an effect on a larger program which should therefore achieve stronger suppression likely to be effective at preventing epidemic dengue in a very wide range of transmission settings, if not all. One caveat is the possible role of other potential vectors such as *Aedes albopictus*. However, *Ae. albopictus* is thought to play a relatively minor role in dengue transmission[48]. Furthermore, there is no obvious reason why similar sterile-male methods should not be developed for this mosquito, a significant vector of chikungunya, if needed. Genetic transformation of *Ae. albopictus* has already been achieved[49].

During this period, the average fluorescence ratio was approximately 12%. This is equivalent to the release of fully-competitive males at 0.14:1, which is at the low end of the range of estimates from modelling (0.15-1.32)[12]. This suggests that the number of engineered sterile male mosquitoes needed to suppress a target wild population may not be as high as we originally, conservatively, estimated.

The degree of suppression attainable in a small-scale trial is limited by immigration[50-52]; Area A is directly adjacent to Area B, which continued to have a roughly 50% ovitrap index. Furthermore, *Ae. aegypti* eggs can remain viable for several months so we expect some adults to continue to emerge from eggs deposited before significant population suppression was achieved. These issues would be less significant, or insignificant, for a larger, longer release program, e.g. operational use.

Long-term egg survival may also explain the apparent residual effect of treatment. The population survives the winter dry season primarily as eggs, then re-expands when the rains return. In effect, the population had little opportunity to re-expand for a period after releases ceased due to seasonal reduction in rainfall. The ovitrap index in Area A remained highly significantly ($p=0.0009$) lower than in Area C in January to June 2011, up to 8 months after the last release (mean ovitrap index: Area A = 5.1% (95% CI: 2.8 – 7.3%) Area C = 17%, (95% CI: 12 – 21%), **Fig. 2c**). The population in Area A was substantially reduced by the release of OX513A RIDL males; the observed reduction in ovitrap index and eggs per trap indicates fewer eggs deposited, which we would expect to translate to a reduced overwintering egg bank. In any case the target population has been slow to return to control levels. This suggests that control by this method could have relatively long-term beneficial effects, and may also be relatively insensitive to brief interruptions in program operations. This effect would likely be even stronger in a larger program with less possibility of immigration.

Methods

Field site selection: The field site in Grand Cayman – the largest of the Cayman Islands – was selected on the basis of biological and other criteria, e.g. presence of *Ae. aegypti*, and preliminary data from small-scale release experiments[4]. Dengue is rare on Grand Cayman, but common in the region and an ongoing threat while *Ae. aegypti* is present. High levels of insecticide resistance in *Ae. aegypti* in Grand Cayman[53] and neighbouring

regions highlight the need for alternative methods of control. The estimated minimum RIDL male release rate – 3150 male/ha/week – required for population collapse might be considered a relatively high number, and reflects the high local mosquito density and the lack of any conventional control at the site.

Release period: 07 May 2010 to 15 Oct 2010. Within this, Period 1 was 7th May to 21th June; Period 2 was 24 June to 30 July; Period 3 was 2 August to 15th October. The release site was East End, approx Lat 19.2089 Lon -81.1055, **Fig 1a,b**). Data were also collected from an untreated control site – Bodden Town, a similar settlement approximately 14 km to the west of East End. Importation and release of mosquitoes was conducted under permit from the Cayman Islands Dept of Agriculture; MRCU activities are also governed by the Mosquito Research and Control Act (2007 Revision). No formal *Ae. aegypti* control measures were implemented at East End in 2010 but aerial and vehicle-based spraying was occasionally used to control a salt-marsh mosquito, *Aedes taeniorhynchus*.

Source of released material: OX513A-Aae, the OX513A insertion ($LA513A^{12}$) in *Ae. aegypti*, was introgressed into a Mexico-derived genetic background by five generations of back-crossing, then made homozygous. Homozygous OX513A eggs were transferred from Oxitec and reared to pupae at MRCU, then mechanically sorted to remove females[54-55]. For quality control, ~1,800 male pupae from each batch were examined under a dissecting microscope prior to release to ensure each batch was >99% male, i.e. <1% females were present in any given batch. In practice we achieved significantly better sorting efficiency; in aggregate only 69 females found out of 104,839 pupae examined during all the per-batch quality control analysis (0.066% female, corresponding to ~ 1 female per 1500 males released, 95% CI: 0.050% - 0.081%). Separated female pupae were rendered non-viable by freezing.

Community engagement: An extensive community engagement program, to be described in detail elsewhere, was undertaken in relation to the biological experiments reported here. Information about the project was provided via the media (print and TV, e.g. http://www.youtube.com/watch?v=_nY_AlWe5kM), written material and by personal contact and briefing. In addition to securing the necessary regulatory approval, local politicians, community leaders and relevant government departments (including ministry representatives from District Administration, Works and Gender Affairs (under which the MRCU operates), The Ministry of Health, Environment, Youth, Sports and Culture, Public Health, The Department of Agriculture and The Department of the Environment) were briefed and consulted prior to and during release activities. Project personnel were present on-site approximately five days per week to provide information and conduct operations. All equipment, vehicles and personnel were clearly marked; equipment and written material included contact information. Permission was sought and received from individual householders prior to project activity on their property, e.g. placement and servicing of BG-Sentinel traps. Community views and concerns were solicited by these routes; direct contact with on-site project staff proved the richest source. The only consistent project-related criticism from the community related to nuisance from the large numbers of males in each individual release in the first part of Period 3. In response we promptly reduced these numbers and moved the release points further from habitations. We also partly substituted with pupal releases, from which

adults emerge over a period of time. Following these changes, no further adverse comments were received.

In principle, increased engagement activity could lead to one or more of a range of possible behavioural changes. For example, residents might more or less actively remove breeding sites, pay more or less attention to mosquito bites, suspected dengue cases, etc. In practice, control activities and associated communication activities are a feature of life on Grand Cayman. Furthermore, communication activities and many program activities (e.g. setting and recovering traps) were undertaken in all areas, and are therefore unlikely to account for the differences between treated and untreated areas observed.

Regulatory affairs: Although the Cayman Islands is a British Overseas Territory, it is not part of the European Union (EU), is not subject to EU Law (Directive 2001/18/EC) regarding the deliberate release of genetically modified organisms, and has autonomous decision making in this area. MRCU has a mandate from Parliament to conduct research activities for mosquito control as enshrined in the Mosquito (Research and Control) Law (2007 Revision). Permission was obtained from the Department of Agriculture for the import and release of the mosquitoes, under the Animals Law (2003 Revision), following a pest risk analysis (PRA)[4]. The trial was also conducted in accordance with the provisions in the Cayman Islands draft National Conservation Law. Work conducted at Oxitec similarly complied with all relevant UK legislation.

Release method: Male pupae were placed in release devices (paper cups or 27 litre plastic cages (Bugdorm DP1000, Bugdorm.com) and allowed to eclose. Three times per week (Monday, Wednesday and Friday), these devices were transferred to the field site and opened to allow males to disperse at points with an average linear separation of approximately 70-90 m, i.e. 1.25-2 release points per hectare. Dead pupae and adults remaining in the devices were counted. For adult release, dead pupae were counted the day before release and dead adults in the release device within two hours of returning the device to the laboratory. For pupal release, dead pupae and adults were counted after retrieval of the used pupal release device to the laboratory. Adult release numbers were calculated as the difference between the number of pupae placed in the devices and the number of dead individuals counted. From 30th August, adult releases were supplemented with pupal releases, in which pupae were placed in an open container in the field to eclose in the field. Predation of pupae and emerging adults form ants was mitigated by placing the pupae release container within a water trap. It was not possible accurately to quantify the number of pupae or adults removed by predators (including ants), however few release devices showed signs of ant activity at the time of removal from the field and we infer that predation by ants was likely not a significant factor. Pupal releases, used in a previous study[4], have the potential advantage of asynchronous release of adults, i.e. the adult males emerge over a period of time. In total, an estimated 2,867,000 adults and 504,000 pupae were released into the field environment. Of the pupae deployed, 13.8% were recovered as dead pupae and adults remaining in release devices after retrieval from field deployment of 2-3 days. The maximum number of adults released from the pupal release devices is therefore estimated at 434,000 although may be lower as the effect of predation of mosquitoes prior to exiting the release device, e.g. by ants, was not included in this calculation.

Monitoring: Approximately 24 BG-Sentinel adult traps[56-57] (Biogents.com) and a larger number of ovitraps (1 April - 15 July, 60; 15 July - 14 Oct, 80; 14 Oct - Dec 16, 43) were used in East End each week; an equivalent untreated control site, Bodden Town, was also monitored with 10 BG-Sentinel and 20 ovitraps throughout (no mosquitoes were released at Bodden Town). Within East End 24 traps were distributed across the all areas in Period 1 (Area A, 5 traps; Area B, 11 traps; Area C, 8 traps). In Period 2 traps were redeployed from area C into Areas A (8) and B (16). In Period 3, traps previously in Area B were redistributed between Areas A (16) where treatment continued and Area C (=6) to provide additional reference for sex ratio in untreated areas. Distances between release points and traps are summarised in **Suppl. Table 1**

	Period 1	Period 2	Period 3
Number of release points	80	45	20
Distance (m) from release point to nearest release point, mean (95% CI)	72.1 (67.6-76.6)	66.9 (59.6-74.2)	61.5 (51.3-71.7)
Distance (m) from release point to nearest ovitrap, mean (95% CI)	44.3 (37.1-51.5)	23.5 (18.4-28.5)	18.1 (13.2-23.0)
Distance (m) from release point to nearest adult trap, mean (95% CI)	66.6 (58.4-74.8)	47.1 (40.5-53.7)	45.3 (37.7-52.9)

Suppl. Table 1. Distances between release points and traps.

Ovitraps, which mimic natural oviposition sites[46] were serviced weekly; BG Sentinels weekly or daily at different stages of the experiment. Sentinel trapping continued until 26 October; ovitrapping continued to June 2011. Eggs from ovitraps were hatched under vacuum; the resulting larvae were examined to confirm lack of *Ae. albopictus* and, up to December 16, scored for characteristic red fluorescence due to the DsRed2 marker[58-59] (Clontech Laboratories, Inc) of OX513A (**Fig. 1e**) using a Leica MZ10F epi-fluorescence microscope. *Ae. albopictus* is present on Grand Cayman but considered to be of relatively restricted in prevalence and geographic distribution. In a one-month pre-release baseline study, all eggs were hatched and identified to species; no *Ae. albopictus* were detected. Throughout the release period, non-fluorescent larvae hatched from ovitrap-collected eggs were also reared to adult as an additional ongoing check for the presence of *Ae. albopictus*; none were detected. On this basis, the ovitrap index was calculated based on the number of eggs present, rather than hatched larvae identified as *Ae. aegypti*. This avoids possible variation in the calculated ovitrap index due to inconsistent egg hatch.

Release of males should change the sex ratio. In untreated areas, the BG Sentinel traps caught 0.54 males per female on average. This variation from a 1:1 ratio could be due to differential attractiveness of the traps to males and females, but is likely additionally or

predominantly a result of males' lower life expectancy, and therefore numbers present in the field[60]. Over-flooding ratio in the release area was therefore estimated as $(x - 0.54y)/0.54y$ where x and y are the numbers of trapped males and females, respectively.

Statistical analysis: A bootstrap method was used to obtain the 95% confidence intervals in which traps were resampled with replacement and the 2.5 and 97.5 percentiles of the estimates were reported from 1,000 bootstrap samples. This method robustly reflects any extra-binomial variation in the data due to clustering of OX513A individuals or males within traps. Likelihood ratio tests, in which 2 times the difference in log likelihoods from two models was compared to a chi-squared distribution, were used to test for between-area differences in ovitrap indices assuming the data arose from binomial distributions. For these tests the ovitrap indices were allowed to differ on a daily basis throughout the period of analysis.

References

1 *Papathanos, P. et al. Sex separation strategies: past experience and new approaches. Malaria Journal **8**, S5, (2009).*

2 *Alphey, L. et al. Sterile-insect methods for control of mosquito-borne diseases – an analysis. Vector Borne and Zoonotic Diseases **10**, 295-311, (2010).*

3 *Rendón, P., McInnis, D., Lance, D. & Stewart, J. Medfly (Diptera:Tephritidae) genetic sexing: large-scale field comparison of males-only and bisexual sterile fly releases in Guatemala. Journal of Economic Entomology **97**, 1547-1553, (2004).*

4 *Harris, A. F. et al. Field performance of engineered male mosquitoes. Nat Biotech **29**, 1034-1037, (2011).*

5 *Simmons, G. S. et al. Field Performance of a Genetically Engineered Strain of Pink Bollworm. PLoS ONE **6**, e24110, (2011).*

6 *Catteruccia, F., Godfray, H. & Crisanti, A. Impact of genetic manipulation on the fitness of Anopheles stephensi mosquitoes. Science **299**, 1225-1227, (2003).*

7 *Irvin, N., Hoddle, M., O'Brochta, D., Carey, B. & Atkinson, P. Assessing fitness costs for transgenic Aedes aegypti expressing the GFP marker and transposase genes. Proc Nat'l Acad Sci USA **101**, 891-896, (2004).*

8 *Marrelli, M., Li, C., Rasgon, J. & Jacobs-Lorena, M. Transgenic malaria-resistant mosquitoes have a fitness advantage when feeding on Plasmodium-infected blood. Proc Natl Acad Sci U S A **104**, 5580-5583, (2007).*

9 *Marrelli, M. T., Moreira, C. K., Kelly, D., Alphey, L. & Jacobs-Lorena, M. Mosquito transgenesis: what is the fitness cost? TRENDS in Parasitology **22**, 197-202, (2006).*

10 *Wise de Valdez, M. R., Suchman, E. L., Carlson, J. O. & Black, W. C. A large-scale laboratory cage trial of the Aedes Densonucleosis Virus (AeDNV). J. Med. Entomol. **47**, 392-399, (2010).*

11 *Atkinson, M. P. et al. Analyzing the control of mosquito-borne diseases by a dominant lethal genetic system. Proc Natl Acad Sci U S A **104**, 9540-9545, (2007).*

12 *Phuc, H. K. et al. Late-acting dominant lethal genetic systems and mosquito control. BMC Biology* ***5****, 11, (2007).*
13 *Dye, C. Models for the population dynamics of the yellow fever mosquito, Aedes aegypti. Journal of Animal Ecology* ***53****, 247-268, (1984).*
14 *Alphey, N., Alphey, L. & Bonsall, M. B. A Model Framework to Estimate Impact and Cost of Genetics-Based Sterile Insect Methods for Dengue Vector Control. PLoS ONE* ***6****, e25384, (2011).*
15 *Oxitec Ltd. (unpublished material).*
16 *Esteva, L. & Mo Yang, H. Mathematical model to assess the control of Aedes aegypti mosquitoes by the sterile insect technique. Mathematical Biosciences* ***198****, 132-147, (2005).*
17 *Sucking, D. M., Hackett, J. K., Barrington, A. M. & Daly, J. M. Sterilisation of painted apple moth Teia anartoides (Lepidoptera: Lymantriidae) by iIrradiation. New Zealand Plant Protection* ***55****, 7-11, (2002).*
18 *Baumhover, A. Eradication of the screwworm fly as agent of myiasis. J. Am. Med. Assoc.* ***196****, 240-248, (1966).*
19 *Wyss, J. H. in Area-Wide Control of Fruit Flies and Other Insect Pests (ed K.H. Tan) 79-86 (Penerbit Universiti Sains Malaysia, 2000).*
20 *Dame, D. A. et al. Release of chemosterilized males for the control of Anopheles albimanus in El Salvador. II. Methods of rearing, sterilization and distribution. The American Journal of Tropical Medicine and Hygiene* ***23****, 482-487, (1974).*
21 *Laven, H. Eradication of Culex pipiens fatigans through cytoplasmic incompatibility. Nature* ***216****, 383-384, (1967).*
22 *Dame, D. A., Curtis, C. F., Benedict, M. Q., Robinson, A. S. & Knols, B. G. J. Historical applications of induced sterilisation in field populations of mosquitoes. Malar J* ***8****, S2, (2009).*
23 *Benedict, M. & Robinson, A. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol.* ***19****, 349-355, (2003).*
24 *Klassen, W. & Curtis, C. F. in Sterile Insect Technique. Principles and practice in area-wide integrated pest management eds V.A. Dyck, J. Hendrichs, & A.S. Robinson) 3-36 (Springer, 2005).*
25 *Dengue Vaccine Initiative (DVI). Planning for the Introduction of Dengue Vaccines: Meeting Report of the Americas Dengue Prevention Board. 22 (2012).*
26 *Focks, D. A Review of Entomological Sampling Methods and Indicators for Dengue Vectors. . (2003).*
27 *Xu, C., Legros, M., Gould, F. & Lloyd, A. L. Understanding Uncertainties in Model-Based Predictions of Aedes aegypti Population Dynamics. PLoS Negl Trop Dis* ***4****, e830, (2010).*
28 *Styer, L. M., Carey, J. R., Wang, J.-L. & Scott, T. W. Mosquitoes Do Senesce: Departure From The Paradigm Of Constant Mortality. The American Journal of Tropical Medicine and Hygiene* ***76****, 111-117, (2007).*
29 *Harrington, L. C. et al. Age-Dependent Survival of the Dengue Vector Aedes aegypti (Diptera: Culicidae) Demonstrated by Simultaneous Release–Recapture of Different Age Cohorts. Journal of Medical Entomology* ***45****, 307-313, (2008).*
30 *Ellis, A. M., Garcia, A. J., Focks, D. A., Morrison, A. C. & Scott, T. W. Parameterization and Sensitivity Analysis of a Complex Simulation Model for*

Mosquito Population Dynamics, Dengue Transmission, and Their Control. The American Journal of Tropical Medicine and Hygiene ***85****, 257-264, (2011).*

31 *Maciel-De-Freitas, R., Codeco, C. T. & Lourenco-De-Oliveira, R. Body size-associated survival and dispersal rates of Aedes aegypti in Rio de Janeiro. Medical and Veterinary Entomology* ***21****, 284-292, (2007).*

32 *Muir, L. E. & Kay, B. H. Aedes aegypti survival and dispersal estimated by mark-release-recapture in northern Australia. The American Journal of Tropical Medicine and Hygiene* ***58****, 277-282, (1998).*

33 *Sheppard, P. M., Macdonald, W. W., Tonn, R. J. & Grab, B. The Dynamics of an Adult Population of Aedes aegypti in Relation to Dengue Haemorrhagic Fever in Bangkok. Journal of Animal Ecology* ***38****, 661-702, (1969).*

34 *McDonald, P. T. Population characteristics of domestic Aedes aegypti (Diptera: Culicidae) in villages on the Kenya coast I. Adult survivorship and population size. Journal of Medical Entomology* ***14****, 42-48, (1977).*

35 *Trpis, M. & Hausermann, W. Dispersal and other population parameters of Aedes aegypti in an African village and their possible significance in epidemiology of vector-borne diseases. The American Journal of Tropical Medicine and Hygiene* ***35****, 1263-1279, (1986).*

36 *Buonaccorsi, J., Harrington, L. & Edman, J. Estimation and comparison of mosquito survival rates with release-recapture-removal data. Journal of Medical Entomology* ***40****, 6-17, (2003).*

37 *Craig, G., Jr. Mosquitoes: Female Monogamy Induced by Male Accessory Gland Substance. Science* ***156****, 1499-1501, (1967).*

38 *Gwadz, R. & Craig, G., Jr. Sexual receptivity in female Aedes aegypti. Mosquito News* ***28****, 586-593, (1968).*

39 *George, J. A. Effect of mating sequence on egg-hatch from female Aedes aegypti (L.) mated with irradiated and normal males. Mosquito News* ***27****, 82-86, (1967).*

40 *Gwadz, R. & Craig, G., Jr. Female polygamy due to inadequate semen transfer in Aedes aegypti. Mosquito News* ***30****, 355-360, (1970).*

41 *Young, A. D. M. & Downe, A. E. R. Renewal of sexual in mated female mosquitoes, Aedes aegypti. Physiological Entomology 7, 467-471, (1982).*

42 *Helinski, M. E. H. et al. Evidence of polyandry for Aedes aegypti in semifield enclosures. Am J Trop Med Hyg* ***86****, 635-641, (2012).*

43 *Mayer, D. G., Atzeni, M. G., Stuart, M. A., Anaman, K. A. & Butler, D. G. Mating competitiveness of irradiated flies for screwworm fly eradication campaigns. Preventive Veterinary Medicine* ***36****, 1-9, (1998).*

44 *Vreysen, M. J. B. in Sterile Insect Technique. Principles and practice in area-wide integrated pest management eds V.A. Dyck, J. Hendrichs, & A.S. Robinson) 325-361 (Springer, 2005).*

45 *Shelly, T. E., McInnis, D. O., Rodd, C., Edu, J. & Pahio, E. Sterile Insect Technique and Mediterranean Fruit Fly (Diptera: Tephritidae): Assessing the Utility of Aromatherapy in a Hawaiian Coffee Field. J. Econ. Entomol.* ***100****, 273-282, (2007).*

46 *Silver, J. Mosquito Ecology: Field sampling methods. 3rd edn, (Springer, 2008).*

47 *WHO-TDR. Scientific Working Group Report on Dengue. Report No. TDR/SWG/08, 162 (WHO, Geneva, 2006).*

48 *Lambrechts, L., Scott, T. W. & Gubler, D. J. Consequences of the expanding global distribution of Aedes albopictus for dengue virus transmission. PLoS Negl Trop Dis* ***4****, e646, (2010).*

49 *Labbé, G. M. C., Nimmo, D. D. & Alphey, L. piggyBac- and PhiC31-Mediated Genetic Transformation of the Asian Tiger Mosquito, Aedes albopictus (Skuse). PLoS Negl Trop Dis* ***4****, e788, (2010).*

50 *Alphey, L. et al. Sterile-insect methods for control of mosquito-borne diseases – an analysis. Vector Borne and Zoonotic Diseases* ***10****, 295-311, (2009).*

51 *Benedict, M. Q. & Robinson, A. S. The first releases of transgenic mosquitoes: an argument for the sterile insect technique. Trends Parasitol* ***19****, 349-355, (2003).*

52 *Whitten, M. & Mahon, R. in Sterile Insect Technique. Principles and practice in area-wide integrated pest management eds V.A. Dyck, J. Hendrichs, & A.S. Robinson) 601-626 (Springer, 2005).*

53 *Harris, A. F., Rajatileka, S. & Ranson, H. Pyrethroid resistance in Aedes aegypti from Grand Cayman. Am J Trop Med Hyg* ***83****, 277-284, (2010).*

54 *Ansari, M., Singh, K., Brooks, G., Malhotra, P. & Vaidyanathan, V. The development of procedures and techniques for mass rearing of Aedes aegypti. Indian J Med Res* ***65****, (Suppl) 91-99, (1977).*

55 *Focks, D. An improved separator for the developmental stages, sexes, and species of mosquitoes (Diptera: Culicidae). J Med Entomol* ***17****, 567 - 568, (1980).*

56 *Krockel, U., Rose, A., Eiras, A. & Geier, M. New tools for surveillance of adult yellow fever mosquitoes: Comparison of trap catches with human landing rates in an urban environment. Journal of the American Mosquito Control Association* ***22****, 229-238, (2006).*

57 *Maciel-de-Freitas, R., Eiras, Á. E. & Lourenço-de-Oliveira, R. Field evaluation of effectiveness of the BG-Sentinel, a new trap for capturing adult Aedes aegypti (Diptera: Culicidae). Memórias do Instituto Oswaldo Cruz* ***101****, 321-325, (2006).*

58 *Lukyanov, K. A. et al. Natural animal coloration can be determined by a nonfluorescent green fluorescent protein homolog. J Biol Chem* ***275****, 25879-25882, (2000).*

59 *Matz, M. et al. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat. Biotechnol.* ***17****, 969-973, (1999).*

60 *Ball, T. S. & Ritchie, S. R. Sampling biases of the BG-sentinel trap with respect to physiology, age, and body size of adult Aedes aegypti (Diptera: Culicidae). J Med Entomol* ***47****, 649-656, (2010).*